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In-vitro developmental studies of *Cheilanthes farinosa* (Forssk.) Kaulf. (Pteridaceae)[1]

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Abstract

Developmental pattern of spore germination, gametophyte growth, differentiation, sex ontogeny and development of sporophytes of *Cheilanthes farinosa* were studied in *in-vitro* conditions. The spore germination and prothallial development are found to be of *Vittaria* type and *Ceratopteris* type, respectively. The species seems be a good colonizer in nature. A considerable numbers of sporophytes were produced through intra- and intergametophytic selfing in culture. Sporophyte production efficiency was observed to be 8% in isolate population and 12% in composite gametophyte population. Genetic barriers and over-exploitation for ornamental purposes causes the reduction in the population size. Protection of this taxon in the natural habitat is urgently required as it grows well in barren and disturbed habitats and help in the establishment of colonies of other plants.

Introduction

Cheilanthes farinosa ((Forssk.) Kaulf. is a homosporous fern belonging to the family Pteridaceae (Smith *et al.* 2006). It is a rock-dwelling fern with a global tropical to sub-tropical distribution in warm and dry regions, often growing in small crevices high up on cliffs. The leaves are often densely covered with trichomes (Nayar 1962). During dry periods, the fronds curl-up, but revive in moist conditions. In India, *C. farinosa* is distributed in Western Himalaya and southern India (Chandra 2000). The species is extensively used for ornamental purposes for which the whole plant is collected from its habitat (Nayar 1962). Over-collection and habitat destruction have resulted in the species being threatened. This present study aims to observe the gametophyte development and reproductive biology of this species to get an insight on the genetic status and requirements for establishment of the taxon in the natural habitat.

Materials and methods

Fronds with mature sori of *C. farinosa* were collected from Dhanaulti (alt 2200m, 30°25'N, 78°15'E) (Western Himalaya) during August 2012. Fronds were stored in a desiccator at room temperature for the release of spores in paper packets. Spores were surface sterilized with 2% sodium hypochlorite solution and rinsed thoroughly with double distilled water and sown in the petri-plates in sterilized Parker's macro- and Thompson's

[1] This paper is dedicated to Elizabeth Anne Brown (1956–2013), a botanist at the National Herbarium of New South Wales

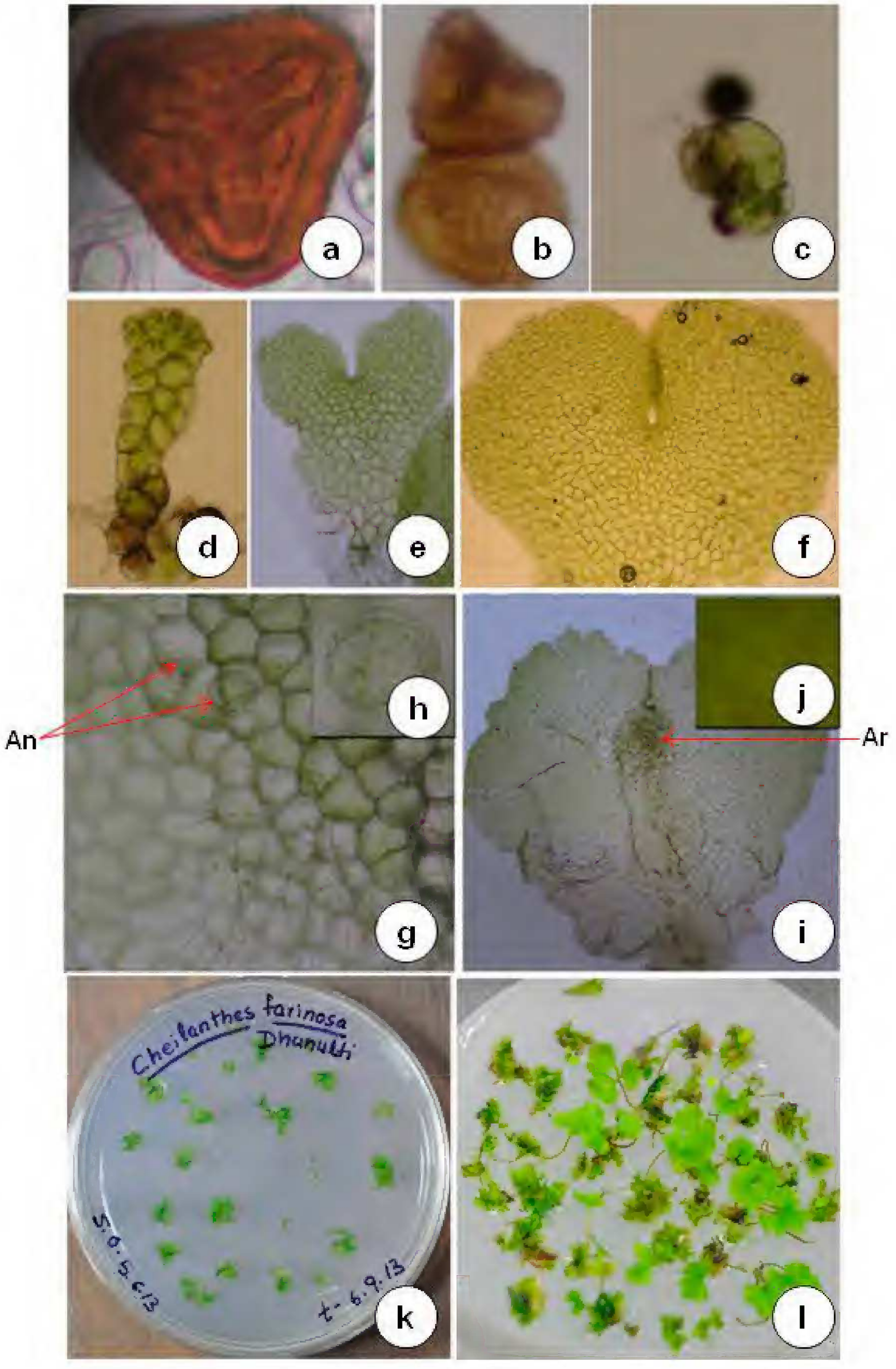

Fig. 1: a, b: Trilete spores; **c:** Emerging protonema and rhizoids; **d:** Two – diamensional stage; **e:** Spatulate stage; **f:** Cordate stage; **g:** Antheridia An -> showing antheridia; **h:** Single Antheridium; **i:** Archegoina Ar -> Archegonia showing below apical notch; **j:** Single Archegonium; **k:** Sporophyte in isolate population; **l:** Sporophyte in composite population

micronutrient culture medium (P&T) gelled with 1% agar. Cultures were kept in a culture room having light intensity ranges between 47.3–56.8 µmol m^{-2} sec^{-1} at 21–25 °C for 16 hour light photoperiod followed by 8 hour dark photoperiod (Klekowski 1969).

Periodically the spore germination and subsequent gametophyte growth, differentiation and sex ontogeny were observed under Olympus Cx21i/Tr microscope and photographed using Olympus Digital Camera. Observations on gametophytes of stock cultures were made and ratios of gametophyte bearing male and female or bisexual conditions were recorded (Table 1). Before initiation of gametangia in stock cultures, the gametophytes were isolated and placed in different petri-plates containing P&T medium in the following manner;

Set 1: 25 petri-plates with single gametophyte in each (isolate culture) and

Set 2: 10 petri-plates with 25 gametophytes in each (composite culture).

After the initiation of the gametangia in stock cultures, watering of all the isolate and composite populations was done from above with sterile distilled water twice a week to facilitate fertilization. Percentage of sporophytes was recorded in both the above mentioned sets (Figs 1k, l).

Five isolate cultures and two composite cultures were kept un-watered throughout the course of the experiment to find out the sexuality of the species. These un-watered populations never produced the sporophytes until the termination of the experiment, thus proved that the species is sexual in nature. These gametophytes were kept as such to observe their regeneration ability.

Table 1: Chronological changes in the sex ratio of a composite culture

Sample size (No.)	Days after sowing	Neuter (No.)	Male (No.)	Female (No.)	Bisexual (No.)
20	60	20	0	0	0
20	70	20	0	0	0
20	80	14	6	0	0
20	90	12	5	0	3
20	100	10	4	2	4
20	110	8	3	4	5
20	120	7	1	5	7
20	130	0	5	9	6
20	140	0	0	15	5
20	150	0	0	20	0
20	160	20	0	0	0

Table 2: Breeding behavior of different populations

Population	Gametophyte studied (No.)	Sporophyte produced (No.)	Sporophyte produced (%)
Isolate (A)	25	2	8
Composite (A x A)	250	30	12

Fig.2: Percentage sporophyte produced in isolate and composite population of *Cheilanthes farinosa*

Results

The spores of *Cheilanthes farinosa* are tetrahedral and aperinous with an average size of 33 x 34 µm (based on measurements of c. 100 spores) (Figs 1a, b). Spore germination was initiated after 7–10 days of sowing and 95% of the spores germinated when sown just 15 days after collection. Spore germination is of equatorial and '*Vittaria* type' (Nayar and Kaur 1971). The spore coat ruptured at the laesura region and the germ filament emerged, generally preceded by the first rhizoid (Fig. 1c). After 20 days, 50% sporelings attained 2-diamensional – stage, but after 35 days, all sporelings attained 2-diamensional stage, out of which 30% spatulate gametophytes were developed (Fig. 1d). After 40–45 days of sowing, 70% cordate and 30% spatulate gametophytes were observed in the culture (Figs 1e, f). Prothallial development is of the '*Ceratopteris* type' (Nayar and Kaur 1971). The viability of spores was totally lost after 12 months when kept at room temperature.

Rhizoids develop at posterior region which were pale brown in colour. Initiation of gametangia started after 70 days of spore sowing. Antheridial initiation was followed by formation of archegonia (Table 1). Antheridia are borne adjacent to the rhizoidal ends of prothalli (Figs 1g, h), while archegonia appear below the apical notch on ventral side. The necks of archegonia are oriented towards the apical notch (Figs 1i, j).

Eighty-five days after sowing, the gametophytes turned bisexual (Table 1). Within 5 months of sowing the spores, the first juvenile leaves appeared in composite population (Figs 1i, j). Only 8% of gametophytes produced in isolate population while 12% gametophytes developed sporophytes in composite population (Table 2, Fig. 2).

Discussion

The populations of *C. farinosa* are continuously decreasing in their natural habitat, due to habitat destruction, deforestation, climate change and unsuitable environment for gametophytic growth and fertilization. Presence of high level of genetic load in the gene pool is reported to be one of the reasons for its rarity (Bir 1987). The taxon is also being exploited for its beauty and extensively used in horticulture. The reproductive biology studies give an insight into the causes of rarity.

Sporophytes were produced only in 12% of gametophytes in composite population and 8% of isolate population, through the fusion of sperm and egg of the same gametophyte. The gametangial ontogeny of this species was found to be more favourable for inter-gametophytic selfing (the fusion of sperm and egg of different gametophytes, both being sibs, i.e. originating from the same parental sporophytes) in comparison to intra-gametophytic selfing (Singh and Roy 1984). Within four months of spore sowing maximum bisexual gametophytes were formed which increases the chance of selfing. After that there was a lower probability of selfing because of the formation of neuter gametophytes.

In composite populations the gametophytes became bisexual and the culture remain male, female and bisexual up to 20 days which provide the opportunity for different types of mating. This may be because the genotypes of the species carried non-allelic recessive sporophytic genes and have larger genetic load present in the gene pool (Klekowski 1969; Ganders 1972).

Majority of taxa having intra-gametophytic selfing and crossing are observed to be successful colonizers and rich in population density (Soltis and Soltis 1992; Korpelainen 1996; Hooper and Haufler 1997; Hereford 2010; Lott *et al.* 2003; Khare *et al.* 2005). For the colonization of open or barren habitat, if spores land sufficiently distant from each other, then only intra-gametophytic selfing is possible. Development of considerable number of sporophytes through intra- and inter-gametophytic selfing is reported in *Microsorum punctatum* (L.) Copel. and *M. alternifolium* (Willd.) Copel. (Srivastava *et al.* 2008; 2014). The similar patterns were also observed in *Asplenium nidus* L., *Lygodium japonicum* (Thumb.) Sw. and *L. flexuosum* (L.) Sw. (Srivastava and Uniyal 2013a, 2013b). The genetic load can affect the relative success of intra- and inter-gametophytic mating events (Srivastava and Khare 2010; Ganders 1972).

Since *C. farinosa* was found to have the capacity to form sporophytes through intra-gametophytic selfing, it may colonise barren land. During the early stages of colonization of new habitat, intra-gametophytic selfing predominates and at later stages mating occurs at the level of inter-gametophytic selfing. After a number of growing seasons the mode of sexuality will transform into almost obligatorily inter-gametophytic selfing.

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